Florida Huanglongbing Science Panel Report

Ben Hill Griffin Auditorium, Rm #1
Citrus Research and Education Center
700 Experiment Station Rd
Lake Alfred, FL

Date: Tuesday, 31 January 2006

The mission of the Florida Huanglongbing Science Panel (SP) is to provide guidance to state and federal officials for a huanglongbing (HLB) response that is based on sound science. Towards that end, scientists met on 31 January, 2006, to provide scientific recommendations on a number of topics. Those in attendance are listed in Appendix I. The meeting was organized by Wayne Dixon, Phil Berger, and Tim Gottwald (see agenda in Appendix I).

Opening comments

David Kaplan stated that we are in need of the best scientifically-based information to best understand what the regulatory response should be to huanglongbing. Based on this information there is a need to move forward with an appropriate response program. The guidance from this science panel will be important to the future development of a regulatory program. Wayne Dixon noted there was a key meeting on Monday (30 January 2006) to further develop the Citrus Health Response Plan (CHRP). Key elements of CHRP have been identified (e.g., nursery and budwood, production practices, packing and processing, harvesting and residential citrus) that will identify minimum regulatory standards for citrus production. It is imperative that standards specific to HLB be integrated into CHRP.

Science Panel Report

The SP was provided a series of topics covering regulatory, control, diagnostic, and survey issues to discuss. The SP addressed high priority issues, and will attempt to address remaining issues at later meetings or conference calls. To put this in perspective, Tim Gast, citrus horticulturist for Southern Gardens Citrus, US Sugar, described the HLB situation in a large commercial grove. Several areas of high disease density with edge effects have been observed. Currently, there are ca. 800 field positive trees out of one million trees. It appears that the disease has been in the grove for at least two or more years. A visual survey for HLB-related symptoms suggests that infection may range from 2 to 34% in some blocks.

The Select Agent (SA) Status of HLB

Because *Candidatus* Liberibacter asiaticus and *Candidatus* L. africanus are select agents, there are additional regulatory challenges and limitations on working with the microbes. The SP addressed two key concerns:

I. There is a desire from the diagnostic community to be able to possess infected tissue (e.g., dried, frozen or lyophilized tissue) so that comparable positive controls are available for diagnostic tests on an as–needed basis. These diagnostic positive controls are essential to achieve and maintain quality and reliability of laboratory-based diagnostics. Current rules state that although a diagnostic lab can accept HLB-suspect samples for testing, any sample proving HLB-positive must be destroyed no less than seven days after testing. To address this, the SP considered whether the pathogen can be recovered from these positive control samples.

   i. There are fundamental characteristics of the HLB pathogen that would indicate that there is little or no risk in allowing diagnostic labs to possess infected tissue that has been processed:
      - The HLB select agent is a fastidious microorganism that has never been cultured.
      - The principle means of pathogen transmission is by an insect vector or by grafting.
      - While theoretically possible to recover the pathogen from processed tissue, this is a highly technical procedure and for which such a recovery has never been demonstrated (see below).

   ii. There are two reports from China indicating that the pathogen was sap-transmitted from citrus to *Cantharantus roseus* (periwinkle) (Li, T. and Ke, C., 2002. Acta Phytophylacica Sinica 29:31-35; Fang et al., 2004. Acta Horticulturae Sinica 31:803-806). While these data suggest that *Liberibacter* spp. might be sap-transmissible, it is important to note that periwinkle is a transmission is accomplished using dodder. The SP was not aware of any reports of mechanical transmission to citrus.

II. Could HLB be used for Bioterrorism purposes?

   a. The panel was asked to consider in a more general sense the status of *Liberibacter* spp. as Select Agents (SA). The SP compared *Liberibacter* spp. to plum pox virus (PPV) because: the two pathosystems are similar; both agents are present in the US and are of relatively limited geographic distribution; both affect woody perennials; and both are vector-borne and graft-transmissible. PPV was once a SA, but has subsequently been removed from the SA list.

ISSUE	PPV	HLB bacterium
• Possibility of the organism to be used as weapon	Low	Low
• Lab research in the absence of its natural vector and only known means of transmission; how easily can it be obtained.	Poses little to no risk to plant health or plant products. Graft and sap-transmissible. Only known to occur in very limited locations in PA – would be difficult to locate in US.	Poses little to no risk to plant health or plant products. Graft-transmissible, but probably not sap-transmissible. It is readily and more easily available from the environment (in S. Florida).
• Transmission mechanisms	Sap-transmissible, and transmission by multiple spp. of native aphids	Probably not sap-transmissible; only by psyllid vector (2 species known, one in US)
• The natural host range is limited largely to plants in one genus	Primarily *Prunus* spp.	Mostly in the *Citrus* genus and some other Rutaceae
• The natural spread of the disease requires insect vectors, and is a complex biological process; artificial spread requires grafting, which is labor intensive, technically demanding and time consuming	Yes. Transmitted naturally by aphids. Intentional aphid transmission (i.e., acquisition and subsequent transmission) Relatively easy to accomplish with suitable source and host material. Graft-transmissible.	Yes. Transmitted naturally by two psyllid species. Intentional psyllid transmission more difficult. Graft-transmissible.
• Spread by pollen or seed.	Not known to occur.	Not known to occur.; under investigation
• Are systemic treatments effective at mitigating the disease	No.	Psyllid control may reduce HLB spread, but not yet published in a refereed paper.
• Destruction of infected trees mitigates the effects of the disease. Removal of the diseased trees and other susceptible hosts removes the source of infection	Yes.	No scientific documentation that tree removal will adequately control HLB.

Considering the ease with which HLB-infected budwood could be obtained from the South Florida environment, the Select Agent status of the HLB pathogen seems unnecessary. The SP does not believe that *Liberibacter* spp. pose a significant risk in terms of use as agents of bioterrorism.

Biological Control

The SP considered the usefulness of biological control of the vector to reduce spread of HLB. Biological control was considered in the context of the entire program, as well as a biological control proposal/scoping document submitted by Dale Meyerdirk (PPQ). The proposal recommended the development of the PPQ facility in Mission, TX as a location

that could provide support of rearing of psyllids and parasites, and efforts aimed at foreign exploration.

While the SP recognizes the inherent value of biological control of insect pests, the value of biological control in this specific pathosystem is quite limited, and may not be a wise use of limited resources: (The SP is aware of current BC efforts in Florida.)

- While biological control and vector reduction in general appears to be a good concept, Asian citrus psyllids have a high reproductive rate which makes effective arthropod biological control a challenge.
- There is no example that the SP was aware of where biological control had an appreciable effect on a vector-borne plant disease. (One possible exception is the HLB infestation on Reunion; however, this is special case since Reunion is an island and therefore a closed ecosystem.) It should also be noted that results of an email questionnaire that was distributed to world experts, as reported to the SP by Dr. Susan Halbert, indicated that all who responded to this question (concerning the potential effectiveness of biological control for HLB) were unaware of any example where psyllid biological control had ever provided economically significant control of an HLB epidemic. (Michaud, 2004. Biological Control 29:260-269; 2002. Entomol. News 113:216-222; 2001. Florida Entomol. 84:608-612.)
- Although there was discussion on the preference for biological control in residential, organic farms and environmentally sensitive areas, the SP had doubts as to whether biological control would significantly slow the spread of disease in these areas.
- In the event the Asian citrus psyllid is observed in the Western States, pursuit of biological control research and development should be reviewed relative to these infestations if the psyllid infestation is determined to be of very limited distribution.
- In general, there may be merit to including cooperators within the research community, leveraging other resources, or finding external sources of funding which would help facilitate future discovery and development of biological control.
- There was consensus that some research effort be put into investigating entomopathogenic fungi which may prove useful as biopesticides.

The overall conclusion of the SP is that the probability of controlling HLB using biological control is low.

Insecticidal control is likely to be more effective than biological control in reducing psyllid populations and reducing disease spread. Control of psyllids and other insects in commercial groves is likely to be addressed only through the use of insecticides, and thus would not be compatible with a biological control strategy. The SP agreed that control

funding, especially from a regulatory standpoint, would be better spent on other, higher priority efforts.

Is removal of trees positive for HLB necessary to stop the disease spread?

The SP discussed tree removal in terms of what to do with trees that test positive for HLB. Currently, the Florida Department of Agriculture and Consumer Services does not mandate removal of HLB-positive trees. Tree owners are encouraged to remove these trees if possible. The removal of diseased trees is viewed in the context of biological and regulatory perspectives: the effect on an epidemic when inoculum is removed or reduced versus the regulatory impact of removing a tree. Although removal of an infected tree will reduce the inoculum available for dispersal, the impact depends, in part, on the overall levels of disease and psyllid vectors in the general area. In other words, if the disease achieves a high incidence, tree removal will not be as effective. From a regulatory perspective, any infected tree in a production system should be quickly rouged, as the producer cannot afford to allow fruit from these trees to be harvested. This is because infected fruit may have an altered undesirable flavor that will potentially taint extracted juice flavor if the proportion of affected fruit is too high. Additionally, infected plants serve as sources for infective psyllids that pose a threat to the remainder of the grove.

Trees that have been exposed to infectious vectors presents a different problem, and recommendations regarding these trees will vary with the situation. At present there is no way to predict if exposed trees are likely to have become infected, nor when they may show symptoms of the disease (see also Diagnostics, below).

Does the SP's consensus of no feasibility of HLB eradication in Florida reached during the November 5, 2005 SP conference call still apply in light of what is known today?

In light of the known current distribution of HLB in 12 counties in FL, the SP reconfirmed that HLB eradication is not feasible. Future scientific emphasis should be directed to meeting the needs of regulatory and educational stakeholders. Key issues identified include whether Murraya (see below) is a true host plant of the pathogens. The scope, extent and benefit of disease quarantine and tree removal needs further discussion. Public outreach certainly deserves further attention as the disease is better understood and management strategies are developed for principal areas of activity (grove production, fruit harvesting, nursery and budwood operations, and residential properties).

Is quarantine in infected areas still necessary?

The SP agreed that commercial nursery stock production should be conducted away from areas of citrus production. A nursery should be quarantined if HLB is found within one mile of the nursery property. In a commercial grove, the SP concluded that a grower will make HLB management decisions based on fruit quality and tree health. Considering the general observations made regarding the disease and the vector: i.e., HLB can be detected in fruit peduncles and psyllids can move on unprocessed fruit, it was agreed that area wide management is important (such as proposed in the CHRP). Currently, in Florida, HLB hosts are prohibited from movement. Host plants of the Asian citrus psyllid are allowed to move if no psyllids are present—i.e., apparently psyllid-free. Some early testing of psyllids collected from *Murraya paniculata* at ornamental nurseries has resulted in at least four HLB- positive psyllids out of 250-1000 psyllids tested. The Division of Plant Industry, Advanced Diagnostics Laboratory has obtained a positive nested PCR for HLB in *Murraya paniculata*; however, a PCR "positive", *i.e*. the presence of *Liberibacter* DNA, does not imply that viable bacteria are present. Further testing and electron microscopy confirmation of *Murraya* infection is ongoing.

Is *Murraya paniculata* a host?

This is an important question, considering it is a host of the psyllid and is a common ornamental plant. Dai et al. (Dai et al. 2005. Research Bull. Plant Protection. Japan 41:53-57; in Japanese), studying the host range of *L. asiaticus* indicated that *M. paniculata* was not susceptible. For their study, they grafted HLB-positive citrus scions onto *Murraya* plants. Subsequently, PCR testing showed no grafted *Murraya* were positive for HLB.

However, recent reports from Brazil indicate that *Murraya* can serve as a host for L. americanus. The SP recommended that this work needs to be repeated with domestic varieties of *M. paniculata* and the Florida isolate of HLB. Dr. Vern Damsteegt indicated that work is underway at Ft. Detrick to experimentally attempt to infect *Murraya* sp. with HLB.

Some early testing of psyllids collected from *Murraya paniculata* at ornamental nurseries has resulted in at least four HLB- positive psyllids out of 250-1000 psyllids tested. It is not certain whether the psyllids acquired the bacteria from the M. paniculata or from other HLB host plants. The Division of Plant Industry, Advanced Diagnostics Laboratory has obtained a positive nested PCR for HLB in *Murraya paniculata*; however, a PCR "positive", *i.e*. the presence of *Liberibacter* DNA, does not imply that viable bacteria are present. Further testing and electron microscopy confirmation of *Murraya* infection is ongoing.

Is there a possibility of seedborne transmission?

There may be no connection between developing seed and the parent plant's vascular system, precluding introduction of the pathogen into seed. However, we do wonder why do seeds abort? Is there perhaps a mobile component produced by the bacteria? These are areas in need of research. Some work is underway in a collaborative effort between Drs. Halbert and Hartung to try to determine if seed-borne transmission of HLB occurs.

What is known about the pathogen/vector relationship?

The pathogen is thought to be multiplicative in the vector. There are definitive experiments that could be done, but these are technically demanding and time-consuming. Clearly, since transmission can occur over the lifetime of an insect, or nearly so, the issue of multiplication in the vector, while of scientific interest, is not particularly relevant in terms of the immediate needs of the program. The issue of whether the pathogen is transmitted transovarially seems more important, considering the risk of inadvertently moving potentially infected psyllid eggs. There are conflicting reports as to whether Liberibacter spp. are transmitted transovarially (Buitendag and von Broembsen, 1993. Proceedings from the 12th Conference of the IOCV 269-273; Roistacher, 1991. *A handbook for detection and diagnosis of citrus and virus-like diseases.* pp. 35-46; van den Berg et al., 1992. Israel Journal of Entomology 25-26:51-56; Xu et al., 1988. Proceedings of the 10th Conference of the International Organization of Citrus Virologists 243-248). Xu et al. (1988) reported that there is no evidence for transovarial transmission, because *D. citri* nymphs collected immediately after hatching on diseased plants did not transmit HLB to indicator plants. It is important to note that the most extensive studies on transovarial transmission of HLB pathogens were done with *T. erytreae*.

van den Berg et al. (1992) allowed immature psyllids to develop on heavily infected plants. When adults emerged, they were allowed to feed and mate on infected plants. After 14 days, the mouthparts of 100 of the females were severed. Ten of these females were placed on each of ten healthy indicator plants, where they laid eggs. Adults from those eggs were allowed to feed on the same plants for 30 days after emergence. Plants were later sprayed, kept insect-free, and tested for HLB after six months. One of the ten plants developed HLB. In another experiment, oviposition was allowed to occur on the infected plants. Crawlers were removed immediately after hatching and prior to feeding and placed on indicator plants. Five of the 24 plants on which these psyllids completed development became infected with HLB. The most logical explanation for these infections is transovarial transmission. However, the authors postulate that the plant in the first experiment could have been infected via oviposition, and those in the second experiment could have been infected as a result of absorption of HLB bacteria from the infected host by the egg. The SP recommended that these experiments should be repeated

with *D. citri* . Dr. Vern Damsteegt indicated that research along these lines is being considered by the ARS in Ft. Detrick.

How far might HLB be dispersed by psyllids?

This is an important question, particularly relative to CHRP and the safety of the Florida citrus nursery industry. The SP was asked if greater than one mile separation between an HLB-infected citrus tree and screened nursery stock is adequate? The short answer is probably not. There is some information indicating that *Trioza* may fly 1-2 km (0.6 – 1.2 mi) and suggestions that the Asian citrus psyllid may do the same. Long distance dispersal has not been documented.

- Circumstantial evidence from observations in Florida suggests dispersal of infectious psyllids of up to 50 miles.
- Work from Brazil indicates HLB dispersal by both insects and movement of infected plants is between 12-15 miles per year.
- There is considerable speculation or circumstantial information on the frequency and distance of psyllid dispersal.
    - In a non-refereed report Aubert (1987) states: "...recent observations suggest possible medium to long transports by strong winds, since upsurges of *D. citri* have appeared in open orchards without windbreak protection, after typhoons (especially Northern Philippines and South East China coast). Passive transport through advective winds seem much more occasional." (Aubert, B, 1987. Proceedings of the Regional Workshop on Citrus Greening Huanglungbin Disease, Fuzhou, China 5pp.)
    - Y. Sakamaki (2005) speculated that the Asian citrus psyllid could move between the Japanese islands of Yoron and Kuyushu, a distance of 270 miles. (Y. Sakamaki, 2005. *Occasional Papers of the Kagoshima University Research Center* 42:121-125.) While admittedly speculative, it is based on the following (paraphrased from Sakamaki's paper):
        - Density- it is well-documented that insects disperse when they surpass peak densities. Peak density for the psyllid has been calculated (by Sakamaki) at 1 adult per new shoot since each female lays about 626 eggs (Tsai and Liu, 2000. Journal of Economic Entomology 93:1721-1725). Any higher than that and they should disperse. From studies by Hayashikawa and Torigoe (2004) on Amami Island, peaks were often reached numerous times a year, leading to many opportune times for dispersal events.
        - Wing structure- The flying muscles of a strong flyer, the vegetable leafminer, was compared with the Asian citrus psyllid. The surface area of the flight muscles is much smaller for the psyllid than the dipteran. Also, the wings of the psyllid are much larger (and both are used) and have a relatively low stroke frequency, like

the brown plant hopper, suggesting that the psyllid passively soars in wind currents much like a kite. Thus, the citrus psyllid is very similar to the brown plant hopper in this respect. They are both basically the same size, and are weak fliers. There is considerable evidence demonstrating the brown plant hopper's ability to migrate large distances (Mills et al., 1996. Bulletin of Entomological Research 86:683-694), and so it is reasonable to believe that the psyllid could similarly disperse over great distances.

- Psyllid migration within Yoron Island follows seasonal wind patterns and moves from south or west, to north or east during June to August, and moves in the completely opposite direction when the wind changes in February to March, and October. (How this was determined was not stated.)
- Jet stream (geostrophic) winds. If the psyllid population density is high, psyllid adults may disperse. They could be taken up into the jet stream which can carry them from Yoron to Kyushu which is about 270 miles away in approximately 17 hours. The psyllid was probably introduced to Yoron Island by human activity, but may spread between islands through wind dispersal.

- It is worth noting that other psyllids (not ACP) have been observed at altitudes of 150 to 7,550 ft.
- By analogy, droplets of water with citrus canker bacteria have been known to disperse up to 35 miles and cause infection.
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It is the opinion of the SP that nursery stock produced within any proximity to possible populations of infectious vectors are at risk. However, there is no scientific information to allow the SP to make recommendations on a 'safe' distance. There is no guarantee that greenhouses or screen houses will provide complete protection from psyllid incursion. In general, an insect-proof structure is next to impossible to achieve. Stock for budwood, which is being moved to north FL, is especially at risk. At present, the nursery industry has expressed it has little confidence in being able to produce HLB disease-free stock. However, the SP recognizes there is a need to assist in the development of regulatory options to achieve the production of certified nursery stock.

Clearly, there is a need for more information on disease epidemiology and vector biology.

What is the drop dead date for nurseries in FL with open air trees to stop selling?

Our current understanding of disease spread and difficulty of detecting presence or absence of the disease with certainty suggests that field grown nursery stock should be viewed as high risk for disease spread into uninfested areas.

What chemical control strategies should be used in commercial groves?

UF/IFAS is currently in the process of developing recommendations on what products could be used for psyllid control. There is one soil-applied systemic insecticide, aldicarb (Temik), available and a number of other chemical controls that may be applied to mature trees. However, it is unknown if there is a direct link between insecticide use and reduction of the spread of HLB. It was noted that imidacloprid (Admire) is approved for use on small trees and containerized material. The same material used on large trees exhibits translaminar flow and needs to be sprayed at 14-day intervals. The SP agreed that abandoned/unmanaged citrus groves present specific problems and will need regulatory attention. It is critical that feral citrus in Florida be eradicated as soon as possible. This is not only important for the management of HLB in Florida, but also for other citrus pests and pathogens including canker, Medfly, and Mexfly. The SP also learned that research is underway at the UF Citrus Research and Education Center on the effects of imidacloprid on psyllids. Similarly, Dr. Vern Damsteegt is conducting an experiment to determine whether infective psyllids can transmit HLB to citrus plants protected by imidacloprid. The SP agreed that abandoned/unmanaged citrus groves present specific problems and will need regulatory attention. Psyllid control may need to be truly area-wide in order to have an effect on disease incidence.

What is the usefulness of sampling psyllids for survey purposes?

It has been suggested that collecting psyllids and assaying them for the presence of the pathogen may be a useful survey tool, particularly in areas where the vector is present, but the disease has not yet been observed. Psyllid sample compositing was discussed; however, the SP is unaware of any data on this technique. It may be an unanswerable question, as a properly controlled experiment would be technically difficult, and there is no way to control the amount of bacteria obtained by individual vectors.

While this seems, at first glance, a good way to at least determine that the pathogen might be present in a particular area, it would likely provide limited epidemiological information. Prior work has indicated that the proportion of infected psyllids in an area is in direct proportion to the incidence of HLB-positive trees in the area. The proportion of psyllids that are sampled relative to the proportion of citrus trees that will be examined is enormously different. At best, even if insects were sampled in groups of perhaps 100 (there is no data to prove that one can reliably detect one infected individual out of 100), in terms of a National Survey (and in Texas, where the vector is known to occur) only an infinitesimally small proportion of the psyllid population will be sampled. Thus, the SP does not recommend that psyllids be surveyed at this time as a survey tool in Texas. It was felt that the system that is currently being used in Florida would be effective

elsewhere, whereby samples that are considered to be highly suspect, on the basis of symptoms, receive priority for diagnostic testing, samples that are medium are next in priority, and so on. Psyllid samples should receive the lowest priority. Also, preliminary research did not discern relationship between highly HLB-positive psyllids and citrus plant expression of disease after psyllid feeding.

There was some discussion of the potential for trapping of psyllids for survey purposes. Yellow sticky traps were observed to be inefficient for psyllid detection. At present, a psyllid-specific pheromone is unknown and it was speculated that the insect may not produce such an attractant. Research is needed to better answer this area of concern.

(Note that the SP was provided estimates of funds available for the upcoming National Survey and limitations that that funding imposes on the number of samples that can be tested.)

What are the current survey activities in Florida?

There is a need from the regulatory standpoint to determine the distribution of HLB in the southern peninsula of Florida. Currently, TDY USDA and DPI contributions have 45 people in the field, and there is another week or so left in this rotation. DPI will continue intensive surveys around nursery sites over the next several months. The question posed to the SP is: should there be continued TDY or intensive survey?

The SP noted that although latency of HLB in a citrus plant means surveys are 1-3 years behind the actual time of disease infectivity, there is still a need to determine disease presence, prevalence, and distribution. Currently, there is a fruit fly trapping network in Florida that could provide an additional opportunity to inspect trees for HLB symptoms. Specific citrus canker/HLB surveys by regulatory agencies are worthy endeavors because citrus canker will likely continue to be a regulated pathogen and therefore surveys should be a part of the developing CHRP. The SP agreed that grove self-inspection surveys would be important contributions to the overall detection of these diseases.

Should efforts be made to develop surveys in packinghouses?

Yes, especially for Texas, Arizona and California. Large portions of the fresh fruit crop pass through packing houses and thus packing houses are good central locations to monitor for symptomatic fruit. Survey resources will need to be identified and in place at the packinghouse to be able to accomplish detection and achieve any trace back to a commercial grove.

What are the diagnostics issues?

- Diagnostics for this pathogen are reasonably robust and sensitive. However, the pathogen is presently patchy in distribution in Florida and may be present at low titer in some citrus samples. While diagnostics are robust for the strain known to occur in Florida, it is unknown how existing assays would perform if new pathogenic *Liberibacter* spp. or pathotypes were present in the US. Primers generic for *Liberibacter* r16S have been developed by the ADL, DPI, that are based upon the presently known species and, in fact, were used for the initial identification of *L. asiaticus* in Florida; however, it is impossible to predict their performance on currently unknown taxa. There needs to be continuous vigilance in looking for different pathotypes of the pathogen.
- Ensuring that as many isolates as possible are collected for maintenance at Beltsville is important, since there is a need to be able to further examine these isolates to determine if different pathotypes are present and to permit investigations into genotypic diversity.
- Research is needed to determine if it is possible to detect an asymptomatic tree. For example, studies on citrus gene expression, facilitated by the use of microarray technology, might identify genetic elements that respond to HLB long before symptoms appear, thus affording the possibility of detecting these plant responses before symptom expression. Applied results of this work could be three or more years away.
- It would be useful to develop antibodies for use in immunoassays, but this is not likely to occur in the foreseeable future.
- As more causal agent genetic information is obtained, the likelihood of improved diagnostic tests increases. Efforts are underway to attempt to sequence the genome of the Florida HLB isolate.
- When is a negative a negative? It is imperative that a proper error analysis of the protocols for the detection of *Liberibacter* in citrus is carried out. As it stands it is not possible to "detect" *Liberibacter* in citrus in any meaningful sense, only to verify the taxonomic the identity of the pathogen. For a given sensitivity threshold, defined by a certified standard, what is the probability that a biologically meaningful titer of pathogen is present for a "negative" defined within the context of that threshold?
- What should be done when samples with strong symptoms result in negative test results?

It is to be expected that bacterial pathogen die-off will occur with the build up of metabolites and decomposition products from both the *Liberibacter* as well as affected citrus tissue. This situation is currently under investigation by the ADL, DPI. The SP recommends in situations as indicated above, that all tests/assays available be used on the sample. Any sample that has strong symptoms yet produces

negative results or results that are ambiguous should be sent immediately to Drs. John Hartung and Yongping Duan for further study.

Literature

Li, T. and Ke, C., 2002. Acta Phytophylacica Sinica 29:31-35

Fang, D., Ganjun, Y., Guoping, W., 2004. Acta Horticulturae Sinica 31:803-806

Michaud, J. P., 2004. Biological Control 29:260-269

Michaud, J. P., 2002. Entomol. News 113:216-222

Michaud, J. P., 2001. Florida Entomol. 84:607-612

Dai, K., Ikeshiro, T., Matsuura, T., Kimura, S., Hamagami, A., Fujiwara, Y., Kobashigawa, Y., Miyakuni, S., 2005. Res. Bull. Plant Prot. Japan 41:53-57

Buitendag, C. H., and von Broembsen, L. A., 1993. Proceedings from the 12th Conference of the IOCV 269-273.

Roistacher, C. N., 1991. *A handbook for detection and diagnosis of citrus and virus-like diseases.* FAO, Rome pp. 35-46.

van den Berg, M. A., van Vuuren, S. P., Deacon, V. E., 1992. Israel Journal of Entomology 25-26:51-56

Xu, C. F., Xia, Y. H., Li, K. B., Ke, C., 1988. Proceedings of the 10th Conference of the International Organization of Citrus Virologists 243-248.

Aubert, B, 1987. Proceedings of the Regional Workshop on Citrus Greening Huanglungbin Disease, Fuzhou, China 5pp.

Sakamaki, Y. 2005. Occasional Papers of the Kagoshima University Research Center 42: 121-125.

Tsai, J. H., Liu, Y. H., 2000. Journal of Economic Entomology 93:1721-1725.

Mills, A. P., Rutter, J. F., Rosenberg, L. J., 1996. Bulletin of Entomological Research 86:683-694.

Florida Huanglongbing Science Panel

Ben Hill Griffin Auditorium, Rm #1

Appendix I

Citrus Research and Education Center
700 Experiment Station Rd
Lake Alfred, FL

Date: Tuesday, 31 January 2006
Time: 8:45 AM – 5:00 PM

Mission: To provide guidance to state and federal officials for a huanglongbing response that is based on sound science

Expectations for the Day's Work: Report on recommendations and action items

Introductions
Science Panel Membership
Organization – Chairman(s), Subcommittees
General Session
Subcommittee Breakouts: no later than 10:00 AM
Lunch
Presentation and Review of Subcommittee Recommendations and Action Items
Report Assignments
Adjourn

Science Issues

The following is not an all-inclusive list, more it is to show the complex science challenge of the huanglongbing, vector, and host complex.

Diagnostics
• Improvements/advances in disease diagnostics (field and laboratory)
Detection of disease low titer
Certainty of disease and strain diagnostics
Rapid diagnostics in field and laboratory
When is a negative a negative?
• Strategy to detect new strains of HLB at incipient field levels
• Molecular biology
Sequencing, culturing, genetics and genomics
Transgenics

Survey
• Survey methodologies for effective and efficient detection and delimitation of HLB in multiple hosts and environmental/host settings (commercial grove, nursery, retail outlets, residential, Asian farms)

Appendix I

Host plants with and without symptoms
Psyllids – trapping, pheromones, baits
Nymphal populations vs. adult populations
    - Seasonal phenology of disease expression
- Data needs for short-term and long-term analyses of disease epidemiology and vector movement
- Inspection frequency

Control
- Strategies for removal of positive and nearby (exposed, latent, etc.) trees
- Best management practices (BMP's) for groves and nurseries where HLB occurs and for where it is not known to occur
- Chemical controls and strategies for adult and nymphal psyllids on (large) trees in groves, nurseries and residential trees
- Biological control of psyllids in different host settings: grove, nursery, residential
- Foreign exploration for biocontrol agents

Regulatory
- Testing of potential host plants: *Murraya paniculata et al.*
- Movement of plants such as *Murraya*'s and Calamondins
- Development and maintenance of host plant list
- Host plants: discernible field symptoms vs. initial colonization
- Insecticides for phytosanitary permits: efficacy and residual toxicity relative to plant shipments
- Labeled product availability for groves, nurseries, retail and residential
- Quarantine strategies in areas of diagnosed HLB
- Movement of fruit: seed transmission, psyllids
- Determination of whether bacteria multiply in psyllids and occurrence of transovarial transmission
- Performance of Koch's Postulates

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Appendix I

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